



(2)

AD-A207 233

Institute Report No. 335

Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in Rats

Larry D. Brown, DVM, LTC, VC

John R.G. Ryabik, BS, SP4

Conrad R. Wheeler, PhD

and

Don W. Korte, Jr., PhD, MAJ, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

DTIC
ELECTED
APR 28 1989
S E D

March 1989

Toxicology Series: 136

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

This document has been approved
for public release and makes no
restriction on distribution or copying.

Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in Rats (Toxicology Series 136)
--Brown *et al*

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. (AR 360-5)

Edwin S. Beatrice 21 Mar 89
Edwin S. Beatrice (date)
COL, MC
Commanding

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

Form Approved
OMB No. 0704-0188

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE		APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
Institute Report No.: 335			
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology	6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory	
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800		7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
9c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012		10. SOURCE OF FUNDING NUMBERS	
11. TITLE (Include Security Classification) (U) Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in Rats	PROGRAM ELEMENT NO 62720	PROJECT NO. A835	TASK NO. AB
12. PERSONAL AUTHOR(S) LD Brown, JRG Ryabik, and DW Korte, Jr.	WORK UNIT ACCESSION NO. DA303913		
13a. TYPE OF REPORT Institute	13b. TIME COVERED FROM 1 MAY 85 TO 5 JUN 85	14. DATE OF REPORT (Year, Month, Day) January 1989	15. PAGE COUNT 71
16. SUPPLEMENTARY NOTATION Toxicology Series No. 136			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Acute Oral Toxicity, DEGDN, Diethyleneglycol Dinitrate, Rat, Mammalian Toxicology, Propellant 1 or -)	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The acute oral toxicity of the nitrate ester, diethyleneglycol dinitrate (DEGDN), was determined in male and female Sprague-Dawley rats by using the oral gavage single-dose method. The median lethal dose (MLD) was 990.4 ± 30.0 mg/kg for male rats and 753.1 ± 35.9 mg/kg for female rats. Clinical signs produced by DEGDN included twitching, tremors, hypertonia, squinting, lacrimation, depression of grasping and righting reflexes, jumping, increased startle reflex, ataxia, cyanosis, inactivity, and prostration. The extent of the neurotoxic component of this clinical signs profile suggests that DEGDN possesses additional pharmacological properties than those routinely associated with the nitrate esters. The duration of clinical signs was acute. Most animals were exhibiting signs by 2 hours after dosing and had either died or cleared by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place DEGDN in the slightly toxic class. Propellant. Propellants. (Rw)			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL EDWIN S. BEATRICE, COL, MC		22b. TELEPHONE (Include Area Code) (415) 561-3600	22c. OFFICE SYMBOL SGRD-ULZ

ABSTRACT

The acute oral toxicity of the nitrate ester, diethyleneglycol dinitrate (DEGDN), was determined in male and female Sprague-Dawley rats by using the oral gavage single-dose method. The median lethal dose (MLD) was 990.4 ± 30.0 mg/kg for male rats and 753.1 ± 35.9 mg/kg for female rats. Clinical signs produced by DEGDN included twitching, tremors, hypertonia, squinting, lacrimation, depression of grasping and righting reflexes, jumping, increased startle reflex, ataxia, cyanosis, inactivity, and prostration. The extent of the neurotoxic component of this clinical signs profile suggests that DEGDN possesses additional pharmacological properties to those routinely associated with the nitrate esters. The duration of clinical signs was acute. Most animals were exhibiting signs by 2 hours after dosing and had either died or cleared by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place DEGDN in the slightly toxic class.

KEY WORDS: Acute Oral Toxicity, Diethyleneglycol Dinitrate, DEGDN, Mammalian Toxicology, Propellant, Rats

Accession For	
NTIS GRA&I <input checked="" type="checkbox"/>	
DTIC TAB <input type="checkbox"/>	
Unannounced <input type="checkbox"/>	
Justification	
By _____	
Distribution/ _____	
Availability Codes	
Dist	Avail and/or
	Special
A-1	



PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Command
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 84017

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: LTC Larry D. Brown, DVM, VC
Diplomate, American College of
Veterinary Preventive Medicine,
American Board of Toxicology

CO-PRINCIPAL INVESTIGATOR: SPC John R.G. Ryabik, BS

PATHOLOGIST: LTC Lance D. Lollini, DVM, MS, VC, Diplomate,
American College of Veterinary Pathologists

DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Diethyleneglycol Dinitrate

INCLUSIVE STUDY DATES: 1 May - 5 June 1985

OBJECTIVE: The objective of this study was to determine the acute oral toxicity of diethyleneglycol dinitrate in Sprague-Dawley rats.

ACKNOWLEDGMENTS

SSG James D. Justus, BS, SP4 James J. Fischer, and SP4 Scott L. Schwebe provided research assistance; SP4 Paul B. Simboli, BS, provided chemical preparation and analysis; Richard A. Spieler and Charlotte L. Speckman provided animal care and facility management; Colleen S. Kamiyama and Ann L. Wilkinson provided secretarial assistance. LTC Larry V. Brown, VC, served as the LAIR Project Director for the acute toxicity studies on DEGDN.

**SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS
INVOLVED IN THE STUDY**

We, the undersigned, declare that study number 84017 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

DON W. KORTE, 3 Jan 89
DON W. KORTE JR., PhD / DATE
MAJ, MSC
Study Director

LARRY D. BROWN, 11 Jan 89
LARRY D. BROWN, DVM / DATE
LTC, VC
Principal Investigator

John R.G. Ryabik, 3 Feb 89
JOHN R.G. RYABIK, BS / DATE
SP4, USA
Co-Principal Investigator

YVONNE C. JOHNSON, 3 Feb 89
YVONNE C. JOHNSON, BS / DATE
DAC
Data Manager

Conrad Wheeler, 3 Feb 89
CONRAD WHEELER, PhD / DATE
DAC
Analytical Chemist



DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

10 March 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84017

1. This is to certify that in relation to LAIR GLP Study 84017, the following inspections were made:

06 March 1984 - Protocol Review
17 May 1985 - Weighing/Dosing

2. The institute report entitled "Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in Rats," Toxicology Series 136, was audited on 27 January 1989.

Carolyn M. Lewis
CAROLYN M. LEWIS, MS
Diplomate, American Board of Toxicology
Chief, Quality Assurance

TABLE OF CONTENTS

Abstract	i
Preface	iii
Acknowledgments	iv
Signatures of Principal Scientists	v
Report of Quality Assurance Unit	vi
Table of Contents	vii
 INTRODUCTION	1
Objective of Study	1
 MATERIALS	1
Test Substance	1
Vehicle	2
Animal Data	2
Husbandry	2
 METHODS	2
Group Assignment/Acclimation	2
Dose Levels	3
Compound Preparation	3
Chemical Analysis of Dosing Solution	3
Test Procedures	3
Observations	4
Necropsy	4
Statistical Analysis	5
Duration of Study	5
Changes/Deviations	5
Storage of Raw Data and Final Report	5
 RESULTS	5
Mortality	5
Lethal Dose Calculations	6
Clinical Observations	9
Gross Pathological Observations	14
 DISCUSSION	14

TABLE OF CONTENTS (cont.)

CONCLUSION	15
REFERENCES	16
APPENDICES	17
Appendix A. Chemical Data	18
Appendix B. Animal Data	25
Appendix C. Historical Listing of Study Events	26
Appendix D. Cumulative Mortality Data	27
Appendix E. Individual Animal Histories	28
Appendix F. Individual Body Weights	51
Appendix G. Pathology Report	62
OFFICIAL DISTRIBUTION LIST	71

**Acute Oral Toxicity of Diethyleneglycol Dinitrate
(DEGDN) in Rats--Brown et al**

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABRDL has tasked the Division of Toxicology, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, a dermal toxicity test in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the acute oral toxicity of diethyleneglycol dinitrate in male and female Sprague-Dawley rats.

MATERIALS

Test Substance

Chemical Name: Diethyleneglycol Dinitrate

Chemical Abstract Service Registry No.: 693-21-0

Chemical structure:



Molecular formula: C₄H₈N₂O₇

Source: Radford Army Ammunition Plant
Radford, VA

Other test substance information is presented in
Appendix A.

Vehicle

The vehicle for DEGDN was corn oil (Sigma Chemical Company, St Louis, MO). The expiration date was April 1995.

Animal Data

Sprague-Dawley rats (Bantin-Kingman Inc, Fremont CA) from a shipment that arrived on 1 May 85 were used in this study. They were identified individually with ear tags. Four rats were submitted as necropsy quality controls. One hundred-eleven rats were dosed in this study. The animal weights on 3 May 85 ranged from 132 to 176 g. Additional animal data appear in Appendix B.

Husbandry

Rats were caged individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow® Diet 5002 (Ralston Purina Company, St Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 23.3 to 25.6°C with a relative humidity range of 41 to 55 percent. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study rats were randomized into 5 dose groups of 10 males and 10 females each. One male vehicle control group of 5 animals was used. Allocation was accomplished using a computer based stratified, weight biased method. The Beckman TOXSYS® Animal Allocation Program was used in conjunction with a Beckman TOXSYS® Data Collection Terminal. The animals were acclimated for 12-19 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of an approximate lethal dose (ALD) determination suggested that the median lethal dose (MLD) was between 500 and 1000 mg/kg. Based on these data, test doses were selected (Table 1).

Compound Preparation

DEGDN was received as a solution containing 18% acetone. The acetone was removed with a rotary evaporator. DEGDN was then suspended in corn oil using a vortex mixer to form a viscous yellow oily suspension. The compound readily went into suspension and there was no discernible separation throughout the dosing procedures.

Chemical Analysis of Dosing Solution

NMR analysis demonstrated that the neat DEGDN is stable for at least 1 year (Appendix A). An emulsion of DEGDN in corn oil was stable for at least 24 hours. Tests for homogeneity of the test compound in the vehicle were conducted. The deviation of individual values from the mean of each set of 3 samples (top, middle, bottom) did not exceed 5% for any suspension.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-36 (3). Animals were fasted overnight before dosing. Volumes of the dosing suspension ranged from 1.98 to 2.79 ml in the males and 1.68 to 2.40 ml in females. The volumes given were based on 10 ml/kg body weight. The dose level was increased by varying the concentration of each suspension. The vehicle control group was given 2.47 to 2.67 ml of corn oil. The dosing was performed by oral gavage without animal sedation or anesthesia. Sterile disposable syringes (Becton, Dickinson & Co, Rutherford, NJ) fitted with 18-gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc, New Hyde Park, NY) were utilized. Animals in Group 1 were dosed between 0951 and 1050 hours on 15 May 1985. Groups 2 and 3 were dosed between 1006 and 1116 on 16 May 1985. After a review of the initial results, Groups 4 through 7 and the six additional animals assigned to Group 1 (females) were dosed between 1019 and 1213 on 22 May 1985.

Table 1: DEGDN Doses

<u>Group</u>	<u>Dose Level</u> (mg/kg)	
	<u>Male</u>	<u>Female</u>
7	N/A	631
1	794	794
4	891	891
2	1000	1000
5	1120	N/A
3	1260	1260
6	(vehicle)	N/A

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were generally performed 1, 2, 4, and 6 hours after dosing and daily for the remainder of the 2-week test period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded weekly during the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those which survived the 14-day study period were submitted for necropsy immediately after sacrifice by barbituate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD₁₀, LD₅₀, and LD₉₀ were derived by probit analysis using the maximum likelihood method, as described by Finney (4). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to plot the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

The study was accomplished according to the protocol and applicable amendments with the following exceptions: the female animals that were originally assigned to the vehicle control group were dosed with 794 mg/kg of DEGDN in order to define more accurately the lower end of the dose range and a cage control group was not used because historical cage control data were already available.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Mortality

Forty-eight animals died as a result of the dosing. Twenty-five deaths (52.1%) occurred within 24 hours of dosing. Fifteen deaths (31.2%) occurred between 24 and 48 hours after dosing. The remaining 8 deaths (16.7%) occurred between 48 and 120 hours after dosing. Table 2 lists the compound related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

TABLE 2: Compound Related Deaths by Group

<u>Group</u>	<u>Dose Level</u> (mg/kg)	<u>Deaths/ Group*</u>	<u>Percent Mortality</u>	<u>Died Within 24 Hours</u>	<u>Died Within 24-48 Hours</u>
MALE					
1	794	0/7	0	0	0
4	891	2/8	25.0	0	0
2	1000	3/7	42.8	2	1
5	1120	7/8	87.5	3	4
3	1260	8/8	100.0	7	1
6	Vehicle	0/5	0	0	0
FEMALES					
7	631	1/9	11.1	0	0
1	794	7/10	70.0	0	2
4	891	7/8	87.5	3	4
2	1000	6/7	85.7	3	3
3	1260	7/7	100.0	7	0

*Number in groups after misdoses removed from study--groups initially had 10 animals; except female Group 1 which had 16 assigned.

Lethal Dose Calculations

Misdosed animals were excluded from statistical analysis and eliminated from the study. Lethal dose values were calculated by probit analysis and the equation for the probit regression line was: $Y = -59.02 \pm 21.37 \log X$ for males and $Y = -31.88 \pm 12.82 \log X$ for females, where X is the dose and Y the corresponding probit value. Figures 1 and 2 graphically present the actual data points and the regression line. Lethal doses calculated from the equation for the probit regression line are presented in Table 3.

Figure 1
DEGDN Dose Response Curve in Male SD Rats

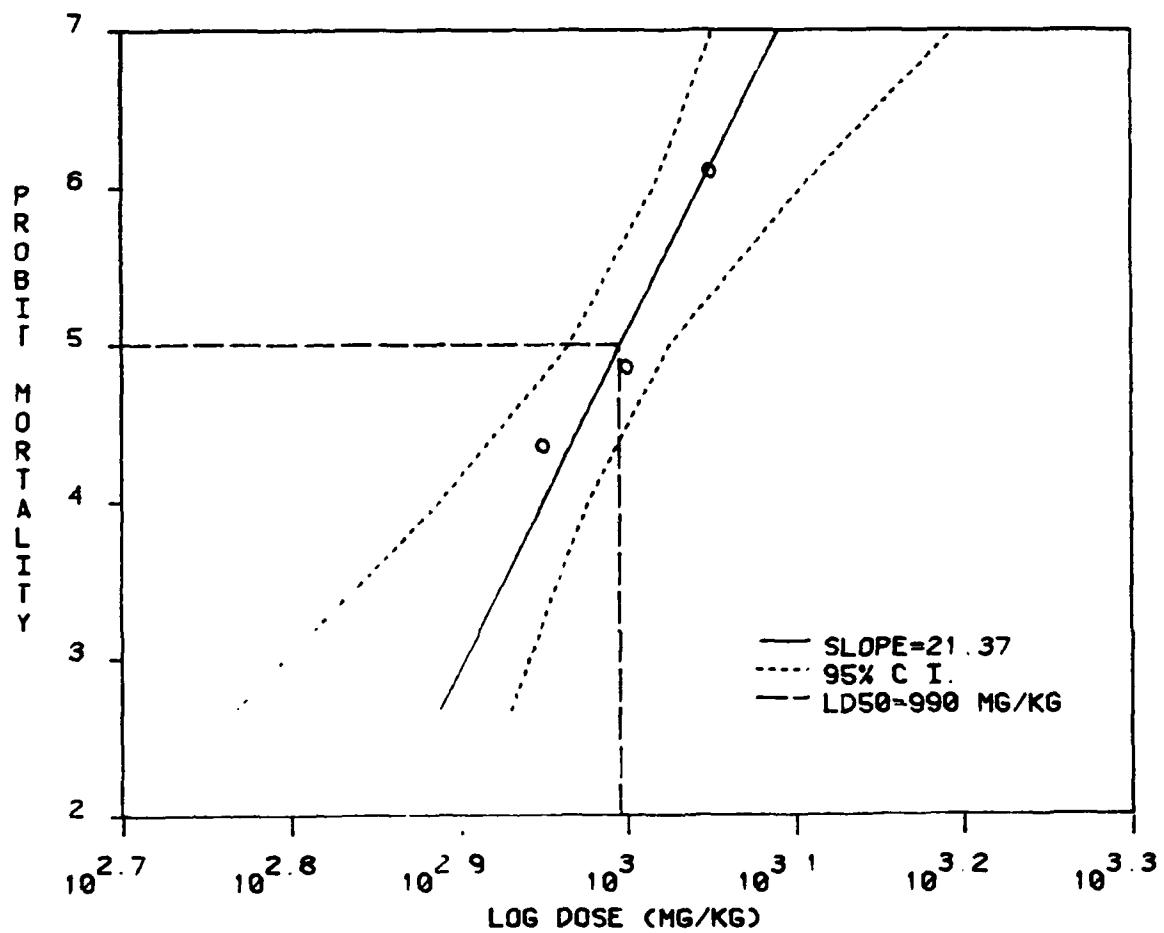


Figure 2
DEGDN Dose Response Curve in Female SD Rats

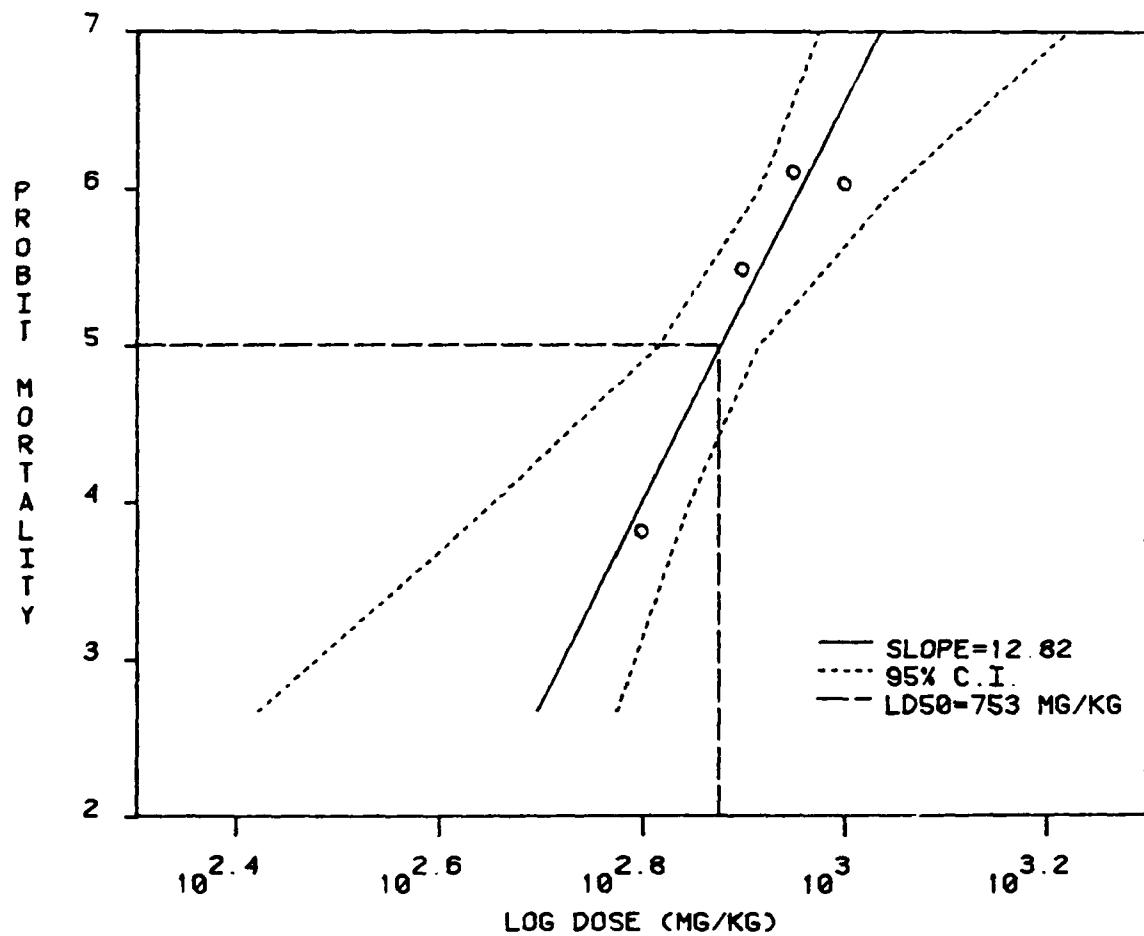


TABLE 3: Calculated Lethal Doses (LD) of DEGDN in Sprague-Dawley Rats

<u>Level</u>	<u>Calculated Dose*</u> (mg/kg)	<u>95% Confidence Limits</u> (mg/kg)
MALES		
LD10	862.6 ± 41.1	(727.9, 924.8)
LD50	990.4 ± 30.0	(923.5, 1060.2)
LD90	1137.0 ± 53.0	(1061.7, 1341.2)
FEMALES		
LD10	598.2 ± 55.9	(402.6, 678.2)
LD50	753.1 ± 35.9	(654.1, 825.5)
LD90	947.9 ± 65.5	(857.9, 1244.8)

* Calculated dose ± standard error.

Clinical Observations

The most frequently observed category of clinical observations was behavioral disturbances (73 of 79 animals dosed). Behavioral signs exhibited by the animals included inactivity, twitching, tremors, hypertonia, irritability, jumping, and ataxia. They were first observed 1 to 3 hours after dosing and, with the exceptions of inactivity and irritability, were no longer observed 72 hours after dosing. Inactivity was last observed 4 days after dosing and isolated incidences of irritability were observed up to Day 8. All animals that died exhibited one or more behavioral signs or had progressed to a moribund condition or death before the first recorded signs. Behavioral signs were present in all dose groups with only the 794 mg/kg male group showing a noticeably lower incidence. Tremors and twitching exhibited an apparent dose response in male animals while the other behavioral signs showed no particular sex or dose response.

The second major category of clinical observations was general signs, and included hunched posture (45 of 79), prostration (40 of 79), and moribund condition (28 of 79).

Hunched posture was also observed in all the control animals. Prostration and moribund condition were observed only in animals receiving the test substance, and in all cases in which these signs were observed, the animals subsequently died.

Ocular signs, which included squinting (49 of 79), lacrimation (24 of 79), and chromodacryorrhea (12 of 79), were observed in a total of 50 of 79 dosed animals with an apparent dose response in the males. However, only lacrimation exhibited a dose response in the female animals.

Another frequently observed category of clinical observations was reflexive signs. Reflexive signs were observed in 46 of 79 dosed animals without any apparent dose response or sex-dependent relationships. Reflexive signs included depressed grasping or righting reflexes and increased startle reflex.

Twenty-seven (14 females and 13 males) of the animals dosed exhibited cyanosis or bluing of the skin, feet, ears, tail, and face. This was first observed 1 to 6 hours after dosing and was absent by 48 hours. Other skin/hair related signs were observed infrequently and included pallor (3 of 79) and rough coat (3 of 79).

Miscellaneous signs was another major category of clinical observations which were observed in 33 of 79 dosed animals. Miscellaneous signs included stains on various areas of the body and abnormally colored urine.

Other clinical signs were observed infrequently and, except for a general paucity of effects in the 794 mg/kg male group, were randomly distributed among the dose groups. Ten animals developed diarrhea. Respiratory signs, which included increased respiratory rate, wheezing, and tachypnea, were observed in 6 animals in the mid-range dose groups. One animal (1260 mg/kg female) died before any clinical signs were recorded. Four males (794 mg/kg) were normal throughout the study. With the exception of the one animal that died very acutely, most clinical signs appeared by 2 hours and cleared by 72 hours after dosing. Tables 4 and 5 contain a summary of clinical observations for males and females, respectively. Appendix E contains individual animal histories.

Weight gains of survivors were not affected by administration of DEGDN. Table 6 presents the mean body weights by groups. Appendix F contains individual weight tables.

TABLE 4: Incidence Summary for Clinical Observations in Male Rats Administered DEGDN

Group Dose (mg/kg)	1 794	4 891	2 1000	5 1120	3 1260	6 Control
Clinical Signs (N=)	7	8	7	8	8	5
General ^a	2	8	6	8	8	5
Behavioral ^b	3	8	7	7	8	5
Diarrhea	0	4	0	0	0	2
Miscellaneous ^c	2	6	2	2	1	4
Skin/Hair ^d	0	1	3	3	8	1
Ocular ^e	1	2	3	7	8	0
Reflexes ^f	1	3	5	7	5	1
Respiratory ^g	0	0	2	0	0	0
Normal Throughout	4	0	0	0	0	0

^a Includes moribund, hunched posture, and prostration.

^b Includes irritable, jumping, ataxia, inactive, hypertonia, tremors and twitching.

^c Includes stains, and abnormally colored urine.

^d Includes pallor, cyanosis, and rough hair coat.

^e Includes squinting, chromodacryorrhea, and lacrimation.

^f Includes depressed grasping and righting reflexes and increased startle reflex.

^g Includes increased respiratory rate, tachypnea, and wheezing.

**TABLE 5: Incidence Summary for Clinical Observations
in Female Rats Administered DEGDN**

Group Dose (mg/kg)	7 631	1 794	4 891	2 1000	3 1260
Clinical Signs (N=)	9	10	8	7	7
General ^a	9	9	8	7	6
Behavioral ^b	9	10	8	7	6
Diarrhea	3	3	0	0	0
Miscellaneous ^c	8	8	2	0	2
Skin/Hair ^d	0	2	4	5	3
Ocular ^e	3	7	7	6	6
Reflexes ^f	4	7	7	5	2
Respiratory ^g	0	2	2	0	0

^a Includes moribund, hunched posture, and prostration.

^b Includes irritable, jumping, ataxia, inactive, hypertonia, tremors and twitching.

^c Includes stains, and abnormally colored urine.

^d Includes pallor, cyanosis, and rough hair coat.

^e Includes squinting, chromodacryorrhea, and lacrimation.

^f Includes depressed grasping and righting reflexes and increased startle reflex.

^g Includes increased respiratory rate and wheezing.

TABLE 6: Mean Body Weights in Grams ± S.E (N)

<u>Dose Groups</u> (mg/kg)	<u>At Receipt</u>	<u>Dosing Day</u>	<u>Midtrial Day</u>	<u>Termination Day</u>
MALES				
794	151.0 ±1.6(7)	216.3 ±2.9(7)	279.1 ±6.8(7)	280.0 ±5.1(7)
891	151.3 ±2.7(8)	248.4 ±5.4(8)	292.7 ±10.2(6)	293.8 ±9.2(6)
1000	157.4 ±1.7(7)	221.3 ±2.8(7)	273.0 ±9.0(4)	288.5 ±6.6(4)
1120	153.3 ±2.0(8)	266.3 ±4.2(8)	--	--
1260	150.4 ±4.7(8)	212.9 ±4.3(8)	--	--
Control	152.0 ±1.3(5)	260.2 ±3.7(5)	319.6 ±3.8(5)	317.0 ±3.9(5)
FEMALES				
631	158.9 ±1.6(9)	205.0 ±3.3(9)	239.3 ±4.6(8)	232.1 ±4.6(8)
794	164.0 ±2.2(10)	198.1 ±4.1(10)	224.7 ±15.3(3)	227.0 ±9.0(3)
891	159.8 ±3.1(8)	214.6 ±5.7(8)	--	--
1000	157.6 ±2.3(7)	181.6 ±3.9(7)	--	--
1260	165.3 ±2.0(7)	192.0 ±1.8(7)	--	--

Gross Pathological Observations

The mortalities which occurred after dosing appear to have been caused by the test compound. A dose-response effect on deaths was apparent in both male and female rats. Multifocal necrohemorrhagic gastritis was observed in 4 animals from Groups 1 (794 mg/kg) and 2 (1000 mg/kg) and is probably treatment related. Liver vacuolization was observed in 7 animals (2 male and 5 female) and may or may not be related to treatment. Renal autolysis was observed in one male and renal necrosis observed in one female. Lymphoid necrosis was observed in one female animal. The veterinary pathologist's report appears in Appendix G.

DISCUSSION

The calculated MLD for DEGDN was 990.4 mg/kg in male rats and 753.1 mg/kg in female rats. These values place DEGDN within the slightly toxic classification (5). Krasovsky et al (6) reported a MLD of 1180 mg/kg in the rat while NIOSH lists the oral MLD of DEGDN as 777 mg/kg in rats (7). Thus, the mortalities observed in this study are within the published range for DEGDN.

DEGDN produced a variety of clinical signs in the rat following oral administration. These signs included tremors, twitching, inactivity, increased startle reflex, squinting, lacrimation, prostration, and cyanosis. Other signs frequently reported, such as hunched posture, irritability, diarrhea, and various stains in the abdominal and perianal regions, were also observed in the vehicle control animals and thus were attributed to administration of the corn oil vehicle.

The number of DEGDN-induced behavioral and reflexive signs was significant as the predominant pharmacological actions of nitrate esters such as nitroglycerin and amyl nitrate are vasodilation and methemoglobin formation (8). Other nitrate esters of military importance such as propyleneglycol dinitrate (PGDN) also produce primarily vasodilation and methemoglobin formation (9). However, Anderson et al (10) also observed a significant neural component to the toxicity profile of triethyleneglycol dinitrate (TEGDN), a nitrate ester similar in physiochemical properties to DEGDN. They reported that TEGDN produced tremors and hyperreactivity to stimuli as well as ataxia and lethargy. They attributed this TEGDN-induced neurotoxicity to the relatively long distance (10 atoms versus adjacent carbon atoms for PGDN) between nitrate groups which gives it

decamethonium-like activity in addition to its nitrate ester actions. DEGDN is similar to TEGDN in that it has seven atoms between its nitrate groups. Thus, the tremors, twitching, and increased startle reflex observed in this study following DEGDN administration could be attributable to a decamethonium-like action similar to that hypothesized for TEGDN.

Another interesting finding was the relatively low incidence of cyanosis (34%) observed following DEGDN administration. Although this low incidence of cyanosis may be related to the difficulty in detecting cyanosis in rodents under artificial (fluorescent) light conditions such as are present in our animal facility, it more likely reflects the fact that DEGDN does not induce methemoglobin formation as readily as the classical nitrate esters. This is supported by Anderson *et al* (10) who reported that at death, animals treated with TEGDN had 30-40% methemoglobinemia versus the 70-80% methemoglobinemia observed in PGDN-treated animals.

CONCLUSION

Diethyleneglycol dinitrate is a slightly toxic compound that produces signs of neurotoxicity in addition to standard symptoms of nitrate ester poisoning. Calculated MLD values were 990.4 ± 30.0 mg/kg in male Sprague-Dawley rats and 753.1 ± 35.9 mg/kg in female Sprague-Dawley rats.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. AD A127846.
2. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, oral toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.
3. Acute oral toxicity study (ALD and LD50). LAIR Standard Operating Procedure OP-STX-36, Letterman Army Institute of Research, Presidio of San Francisco, CA. 15 June 1984.
4. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1971:20-80.
5. Hodge HC, Sternier JH. Tabulation of toxicity classes. American Industrial Hygiene Association Quarterly 1943; 10:93-96.
6. Krasovsky GN, Korolev AA, Shigan SA. Toxicological and hygienic evaluation of diethylene glycol dinitrate in connection with its standardization in water reservoirs. J Hyg Epidemiol Microbiol Immunol 1973; 17:114-119.
7. Talken RL, Lewis RJ Jr. Diethylene glycol dinitrate (ID 6825000). Cincinnati, OH: National Institute for Occupational Safety and Health (NIOSH), 1983; Registry of Toxic Effects of Chemical Substances (RTECS) 1981-82, Vol 2, p 88.
8. Needleman P, Johnson EM, Jr. Vasodilators and the treatment of angina. In: Gilman AG, Goodman LE, Rall TW, Murad F, eds. The pharmacological basis of therapeutics. 7th ed. New York: Macmillan Publishing Co, Inc., 1985; 819-833.
9. Clark DG, Litchfield MH. The toxicology, metabolism and pharmacologic properties of propylene glycol 1,2-dinitrate. Toxicol Appl Pharmacol 1969; 15:69-73.
10. Andersen M, Koppenhaver, RE, Jenkins LJ, Jr. Some neurotoxic properties of triethylene glycol dinitrate: a comparison with decamethonium. Toxicol Appl Pharmacol 1976; 36:585-594.

Appendix A. Chemical Data.....	18
Appendix B. Animal Data.....	25
Appendix C. Historical Listing of Study Events.....	26
Appendix D. Cumulative Mortality Data.....	27
Appendix E. Individual Animal Histories.....	28
Appendix F. Individual Body Weights.....	51
Appendix G. Pathology Report.....	62

Appendix A: CHEMICAL DATA

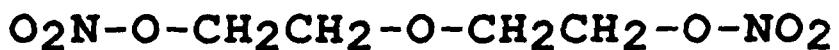
Chemical Name: Ethanol, 2,2'-oxybisdinitrate

Alternate Chemical Name: Diethyleneglycol dinitrate (DEGDN)

Chemical Abstracts Service Registry No.: 693-21-0

LAIR Code No.: TP047

Chemical Structure:



Molecular Formula: C₄H₈N₂O₇

Molecular Weight: 196

Physical State: Pale yellow liquid

Density (g/cm³): 1.38¹

Analytical Data: The compound chromatographed as a single peak (retention time 5.4 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% acetonitrile; flow rate, 0.9 ml/min; detection wavelength, 205 nm.² NMR (300 MHz, CD₃CN): 3.75 δ (complex multiplet, 4H, -CH₂-O-CH₂-), 4.61 complex

¹ Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland; US Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. AD A127846, p. 17.

² Wheeler CR. Toxicity Testing of Propellants. Laboratory Notebook #85-12-023, p. 31. Letterman Army Institute of Research, Presidio of San Francisco, California.

Appendix A (cont.): CHEMICAL DATA

multiplet, 4H, -CH₂ONO₂).³ Additional singlet signals of approximately equal intensity were observed at 2.08 d, and were due to sample impurities. Integration of all signals in the spectrum demonstrated that the sample contained 96.6% DEGDN. The impurities were not identified. IR(KBr): 2896, 1632, 1429, 1390, 1373, 1279, 1139, 1032, 909, 857, 758, 707, 655, 572cm⁻¹.⁴

Stability: The DEGDN was shipped containing 18% acetone (a desensitizer) and arrived at LAIR on 12 December 1984. The acetone was removed by rotary evaporation prior to studies with the propellant. Analysis of the compound one year after it was received gave the results described above. Stability of the compound in corn oil (the dosing vehicle) was examined. As determined by HPLC, the concentration of DEGDN in corn oil emulsions 24 h after preparation was within 1% of the target value.⁵

Source: Radford Army Ammunition Plant, Radford, Virginia
(prime contractor: Hercules Inc., Wilmington, Delaware).

Lot No.: RAD84M001S214

³ Ibid. pp. 44-48.

⁴ Ibid. pp. 49-50.

⁵ Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, pp. 57-60. Letterman Army Institute of Research, Presidio of San Francisco, California.

Appendix A (cont.): CHEMICAL DATA

**Analysis of DEGDN/Corn Oil Emulsions
for Stability, Homogeneity, and Concentration**

INTRODUCTION:

Emulsions of diethyleneglycol dinitrate (DEGDN) in corn oil were prepared by shaking or stirring mixtures of the two components. The emulsions were subsequently used for dosing animals in the GLP Studies #84017 (acute oral toxicity in rats) and #84018 (acute oral toxicity in mice). After dosing, the remainder of each emulsion was stored at 4°C for analysis. Determination of the DEGDN concentration was accomplished by reverse-phase liquid chromatography.

MATERIALS:

Chromatographic analysis was performed using a Hewlett-Packard 1090 high pressure liquid chromatography (HPLC) system with diode array detector (Hewlett-Packard, Palo Alto, CA). Separations were obtained on a Brownlee RP-18 column (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA). HPLC grade acetonitrile and water were obtained from the J.T. Baker Chemical Co., Phillipsburg, NJ.

METHODS:

Analysis of DEGDN solutions was accomplished under the following HPLC conditions: solvent, 70% acetonitrile-30% water; solvent flow, 0.9 ml/min; injection volume, 10 µL; detector wavelength, 205 nm. The HPLC mobile phase was used to prepare standards as well as to extract the DEGDN/corn oil mixtures. Standard solutions of DEGDN ranging in concentrations from 80 to 670 mg DEGDN/ml were prepared in 70% acetonitrile. A set of 12 standards covering this range was analyzed both before and after each set of samples (diluted dosing emulsions).

To measure the effect of corn oil on DEGDN analysis, a series of DEGDN solutions in 70% acetonitrile were prepared with and without the inclusion of corn oil.⁶ Eight solutions of DEGDN at 300 µg/ml were prepared by adding 6 ml aliquots

⁶ Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, pp. 43-48. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

of stock solution (50 mg DEGDN/ml) to 50 ml volumetric flasks. Corn oil (1 ml) was then added to 4 of the flasks before filling all to volume with 70% acetonitrile. One ml from each volumetric flask was transferred to a second volumetric flask for a further dilution prior to analysis.

To determine if the emulsions of DEGDN in corn oil prepared for dosing were homogenous, a series of emulsions were prepared with DEGDN concentrations spanning the range of concentrations employed in the dosing preparations.⁷ Emulsions (15 ml each) containing 50, 150, and 300 mg of DEGDN per ml were prepared in 20 ml scintillation vials. After stirring with a magnetic stir bar for at least 5 min, aliquots from the top, middle, and bottom of the emulsions were removed and transferred to tared 25 ml volumetric flasks. The exact weight of the aliquot was recorded and the flask filled to volume. One ml of this solution was transferred to a second volumetric flask for further dilution prior to HPLC analysis.

To determine the stability of DEGDN in corn oil, an emulsion (100 mg DEGDN/ml corn oil) was prepared.⁸ Eight 1-ml aliquots were removed and transferred to individual tared volumetric flasks. The weights of the aliquots were recorded and the flasks divided into two equal groups. The first set of four was analyzed immediately and the second set 24 h after preparation of the emulsion. For analysis, the flasks were filled to volume with 70% acetonitrile. One ml from each flask was transferred to a second volumetric flask for further dilution prior to analysis by HPLC.

To prepare the dosing emulsions for analysis the DEGDN/corn oil mixtures were removed from the refrigerator and warmed to room temperature. After rapidly stirring each sample for a minimum of 5 min, an aliquot of approximately one ml was removed and transferred to a tared 50 ml volumetric flask. The weight of each aliquot transferred was

⁷ Ibid. pp. 30-40.

⁸ Wheeler CR. Toxicology Testing of Propellants. Laboratory Notebook #85-12-023, pp. 74-75. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

recorded and the flask filled to volume. A second dilution was required prior to analysis by HPLC.⁹

RESULTS

Under the conditions of the analysis DEGDN eluted with a retention time of 4.2 min. A plot of the DEGDN concentration versus peak area was linear within the range of concentrations (80.2-855.5 µg/ml) employed as standards. The differences in peak areas between corresponding standards run before and after the samples were less than 1%. As shown at the bottom of Tables 1, 2, 3, and 4, the equation for the standard plot was virtually identical from assay to assay.

Extraction of the dosing emulsions with 70% acetonitrile-30% water resulted in a very clean chromatogram with no peaks from corn oil. To evaluate the effect of corn oil on DEGDN quantitation the data obtained from analysis of solutions prepared with and without corn oil (Table 1) was analyzed using the t-test. The concentration of DEGDN in the two sets of samples was not significantly different ($p = 0.91$).¹⁰ This demonstrated that corn oil does not affect the results of the assay under the conditions described, and extraction is therefore quantitative.

The data from the assessment of emulsion homogeneity are presented in Table 2. For each emulsion the deviation of concentration determined for the top, middle, and bottom of the emulsion was less than 5% of the mean. Analysis of DEGDN/corn oil emulsions showed that the concentration of DEGDN in an emulsion stored for 24 h at room temperature was 97.3% of value determined immediately after preparations (Table 3). The data obtained from the analysis of dosing emulsions are presented in Table 4. All but two of the values were within 10% of the target. The two values that fall outside this range do so by only 2.3 and 2.5%.

⁹ Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, pp. 48-56. Letterman Army Institute of Research, Presidio of San Francisco, CA.

¹⁰ Ryan T, Joiner B. Ryan B. Minitab Computer Program for the Data General MV/8000, University Park, PA: Pennsylvania State University, 1982.

Appendix A (cont.): CHEMICAL DATA**Table 1.** Analysis of DEGDN with and without corn oil. The target concentration of DEGDN was 300 mg/ml

[DEGDN] by Analysis (mg/ml) *	
Corn Oil	Without Corn oil
292	294
296	301
296	294
297	293
Average 295.2	295.5

Equation of the standard plot, $Y = 0.055X + 0.025$; $r = 0.9998$ **Table 2.** Assessment of homogeneity for DEGDN/corn oil emulsions. Aliquots of approximately 1 ml were withdrawn from the top (T), middle (M), and bottom (B) of the emulsions and analyzed.*

Target [DEGDN] (mg/ml)	Site of Sampling	[DEGDN] Determined by Analysis	Mean [DEGDN] (T+M+B)/3	Deviation from Mean [DEGDN]
49.7	T	48.8	49.0	99.6
	M	49.0		100.0
	B	49.2		100.4
150.0	T	140.3	145.5	96.4
	M	145.0		99.7
	B	151.2		103.9
299.7	T	279.1	290.3	96.1
	M	301.2		103.8
	B	290.5		100.1

*Equation of standard plot: $Y = 0.057 X - 0.309$; $r = 0.9998$

Appendix A (cont.): CHEMICAL DATA

Table 3. Determination of DEGDN stability in corn oil. An emulsion of DEGDN in corn oil was prepared and analyzed immediately after preparation and 24 h later (4 samples were analyzed each time).

Concentration Determined by Analysis (mg/ml)		
Time: 0 Hour*		Time: 24 Hours†
	97.9	96.2
	98.9	95.3
	98.1	95.0
	96.8	94.7
Average:	97.9	95.3

*Equation of standard plot: $Y = 0.058X - 0.138$; $r = 0.9999$

†Equation of standard plot: $Y = 0.058X - 0.187$; $r = 0.9998$

Table 4. Concentration of DEGDN in dosing emulsions prepared for GLP Studies 84017 and 84018. Samples that were analyzed a second time for verification have been denoted with an R (Reanalyzed) in front of the target concentration. In each case reanalysis yielded a value for concentration that was within 3% of the initial concentration.

Study No.	Target (mg/ml)	Date Prepared (1985)	Date Analyzed (1985)	Actual (mg/ml)	% Target
84017	50.0	07 May	22 Nov*	49.1	98.2
	100.0	07 May	22 Nov	102.1	102.1
	(R) 150.0	07 May	19 Nov†	168.5	112.3
	(R) 126.0	14 May	22 Nov	110.3	87.5
	79.4	14 May	22 Nov	81.7	102.9
84018	100.0	14 May	22 Nov	96.4	96.4
	193.0	20 May	19 Nov	194.5	100.8
	164.0	20 May	19 Nov	167.4	102.1
	139.0	20 May	19 Nov	138.0	99.3
	118.0	23 May	22 Nov	121.1	102.6
	100.0	23 May	19 Nov	95.0	95.0

* Equation of standard plot: $Y = 0.059X - 1.449$; $r = 0.9986$

† Equation of standard plot: $Y = 0.056X + 0.010$; $r = 0.9999$

Appendix B: ANIMAL DATA

Species: *Rattus norvegicus*

Strain: Sprague-Dawley

Source: Bantin and Kingman, Inc
Fremont, CA

Sex: Male and female.

Date of birth: Males: 19 March 1985
Females: 13 March 1985

Method of randomization: Weight bias, stratified animal allocation

Animals in each group: 10 male and 10 female animals initially assigned. Five males for control group.

Condition of animals at start of study: Normal

Body weight range at dosing: 168-279 g

Identification procedures: Ear tag

Pretest conditioning: Quarantine/acclimation 3-14 May 85 (12 days) for males and females.

Justification: The laboratory rat has proven to be a sensitive and reliable animal model for lethal dose determinations.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
1 May 85	Rats for GLP Protocol 84017 arrived. Rats were checked for physical condition, sexed, and individually caged.
3 May 85	All rats were weighed and tagged, and 4 rats (2 male and 2 female) were submitted for necropsy quality control.
3-14 May 85	Animals were observed daily in quarantine.
7 May 85	Nineteen rats (ALD) were dosed.
10 May 85	Animals were weighed.
13 May 85	Animals were randomized into dose groups.
15 May 85	Group 1 animals were fasted overnight, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
16 May 85	Group 2 and 3 animals were fasted overnight, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
22 May 85	Groups 1(female repeat), 4, 5, 6, and 7 animals were fasted overnight, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
16 May - 4 Jun 85	All animals were observed daily in a.m. and p.m. for 14 days following dosing.
24,30 May 85	Animals were weighed approximately 7 days (midtrial) after dosing.
29 May 85	All surviving animals in Group 1 were weighed, sacrificed, and submitted for necropsy.
30 May 85	All surviving animals in Groups 2 and 3 were weighed, sacrificed, and submitted for necropsy.
5 Jun 85	All surviving animals in Groups 1 (repeat), 4, 5, 6, and 7 were weighed, sacrificed, and submitted for necropsy.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group)

<u>Dose</u> mg/kg	<u>Animals/</u> <u>Group</u>	Time After Dosing (Days)									
		1	2	3	4	5	6	7	8	9	10-14
MALES											
794	7	0	0	0	0	0	0	0	0	0	0
891	8	0	0	1	2	2	2	2	2	2	2
1000	7	2	3	3	3	3	3	3	3	3	3
1120	8	3	7	7	7	7	7	7	7	7	7
1260	8	7	8	8	8	8	8	8	8	8	8
Vehicle	5	0	0	0	0	0	0	0	0	0	0
FEMALES											
631	9	0	0	0	1	1	1	1	1	1	1
794	10	0	2	6	6	7	7	7	7	7	7
891	8	3	7	7	7	7	7	7	7	7	7
1000	7	3	6	6	6	6	6	6	6	6	6
1260	7	7	7	7	7	7	7	7	7	7	7
TOTAL		25	40	45	47	48	48	48	48	48	48

Appendix E: INDIVIDUAL ANIMAL HISTORIES**MALE: VEHICLE CONTROLS**

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00485	Irritable Hunched Posture	May 22, 23, 28 May 22	Moderate Slight
85D00498	Hunched Posture Irritable Diarrhea Increased Startle Reflex Stain, Perianal, Yellow Rough Coat	May 22-24 May 22-24 May 22 May 22 May 23 May 24, 25	Marked Marked Slight Slight Moderate Slight
85D00510	Irritable Hunched Posture Diarrhea Stain, Perianal, Yellow	May 22, 23 May 22, 23 May 22 May 23	Marked Marked Marked Moderate
85D00516	Irritable Hunched Posture Stain, Perianal, Yellow	May 22 May 22, 23 May 23	Slight Moderate Moderate
85D00526	Irritable Hunched Posture Stain, Perianal, Yellow	May 22-24 May 22-24 May 23	Marked Moderate Moderate

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 794 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00463	Misdose	N/A	N/A
85D00470	Inactive	May 15, 16	Marked
	Tremors	May 15	Slight
	Squinting	May 15	Moderate
	Hunched Posture	May 15	Moderate
	Increased Startle Reflex	May 15	Moderate
	Stain, Perianal, Brown	May 17	Slight
85D00471	Normal	N/A	N/A
85D00484	Normal	N/A	N/A
85D00490	Misdose	N/A	N/A
85D00496	Inactive	May 15	Slight
	Irritable	May 16, 17	Slight
	Stain, Perianal, Green	May 16	Moderate
85D00497	Normal	N/A	N/A
85D00513	Normal	N/A	N/A
85D00517	Inactive	May 15	Moderate
	Hunched Posture	May 15	Slight
	Irritable	May 16	Slight
85D00523	Misdose	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 891 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00459	Hunched Posture Twitching Hypertonia Inactive Tremors Diarrhea Stain, Perianal, Dark	May 22 May 22 May 22 May 22, 23 May 22 May 23 May 24	Moderate Slight Marked Marked Slight Marked Moderate
85D00467	Hunched Posture Inactive Twitching Increased Startle Reflex Hypertonia Stain, Perianal, Yellow	May 22 May 22 May 22 May 22 May 22 May 23, 24	Slight Slight Slight Moderate Marked Marked
85D00481	Hunched Posture Inactive Hypertonia Twitching Squinting Increased Startle Reflex Stain, Mouth/Nose, Brown Diarrhea Stain, Abdomen, Yellow Stain, Perianal, Yellow Death	May 22-24 May 22, 23 May 22 May 22 May 22 May 22 May 23 May 23 May 24 May 24 May 24	Marked Moderate Moderate Moderate Moderate Moderate Slight Marked Moderate Marked 2.2 days
85D00502	Hunched Posture Irritable	May 22 May 22	Marked Slight
85D00504	Hunched Posture Inactive Hypertonia Tremors Irritable Diarrhea Stain, Perianal, Yellow	May 22-24 May 22 May 22 May 22 May 23 May 23 May 23, 24	Marked Moderate Marked Moderate Slight Moderate Marked

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 891 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00506	Hunched Posture Inactive Hypertonia Twitching Squinting Increased Startle Reflex Prostrate Tremors Ataxia Lacrimation Irritable Rough Coat Diarrhea Stains, Abdomen Stains, Perianal Death	May 22-25 May 23, 25 May 22 May 22 May 22, 25 May 22 May 22 May 23, 24 May 23 May 23 May 24 May 25 May 23 May 24, 25 May 23, 24 May 26	Moderate Moderate Slight Moderate Marked Slight Present Slight Moderate Marked Slight Moderate Moderate Marked Marked 4 days
85D00511	Misdose	N/A	N/A
85D00520	Hunched Posture Inactive Irritable Stain, Perianal, Dark	May 22-24 May 22 May 22-24 May 23	Marked Slight Slight Marked
85D00522	Misdose	N/A	N/A
85D00527	Inactive Hunched Posture Irritable	May 22 May 22 May 22	Slight Marked Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1000 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00464	Hunched Posture Inactive Hypertonia Prostrate Depressed Grasping Reflex Tachypnea Irritable Rough Coat Stain, Nose, Red Stain, Perianal, Brown	May 16 May 16 May 16 May 16 May 16 May 16 May 17 May 17-19 May 16 May 17	Moderate Moderate Marked Present Slight Slight Slight Moderate Slight Moderate
85D00468	Hypertonia Prostrate Depressed Grasping Reflex Squinting Twitching Stain, Nose, Red Moribund/Ataxia Death	May 16, 17 May 16, 17 May 16 May 16 May 16 May 16 May 16, 17 May 18	Marked Present Slight Moderate Slight Present 2 days
85D00477	Hypertonia Prostrate Depressed Grasping Reflex Squinting Twitching Tremors Cyanosis Pallor Moribund Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Slight Present Marked Marked Marked Moderate Moderate Slight Present 1 day
85D00478	Hunched Posture Depressed Grasping Reflex Irritable Inactive	May 16 May 16 May 16 May 16	Moderate Marked Slight Moderate
85D00480	Misdose	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1000 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00483	Prostrate Tremors Twitching Squinting Depressed Grasping Reflex Increased Resp. Rate Cyanosis Moribund Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Present Marked Marked Marked Moderate Slight Slight Present 1 day
85D00487	Irritable	May 23, 24	Marked
85D00492	Misdose	N/A	N/A
85D00499	Misdose	N/A	N/A
85D00501	Hunched Posture Tremors Inactive Hypertonia	May 16 May 16 May 16 May 16	Moderate Slight Slight Moderate

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1120 mg/kg DIETHYLENEMEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00466	Hypertonia	May 22	Marked
	Prostrate	May 22	Present
	Increased Startle Reflex	May 22	Marked
	Squinting	May 22	Marked
	Twitching	May 22	Moderate
	Tremors	May 23	Slight
	Cyanosis	May 23	Slight
	Stain, Perianal, Yellow	May 23	Slight
	Moribund	May 23	Present
	Death	May 23	1.2 days
85D00469	Hypertonia	May 22	Moderate
	Prostrate	May 22	Present
	Increased Startle Reflex	May 22	Marked
	Squinting	May 22, 23	Marked
	Twitching	May 22, 23	Moderate
	Stain, Nose, Red	May 23	Slight
	Moribund	May 22, 23	Present
	Death	May 23	1.2 days
85D00473	Hypertonia	May 22	Moderate
	Prostrate	May 22	Present
	Increased Startle Reflex	May 22	Slight
	Squinting	May 22	Marked
	Twitching	May 22	Marked
	Tremors	May 22	Marked
	Moribund	May 22	Present
	Death	May 23	1 day
85D00479	Misdose	N/A	N/A
85D00488	Hypertonia	May 22	Slight
	Prostrate	May 22	Present
	Increased Startle Reflex	May 22	Slight
	Squinting	May 22	Marked
	Twitching	May 22	Moderate
	Tremors	May 22	Slight
	Cyanosis	May 22, 23	Marked
	Lacrimation	May 23	Marked
	Moribund	May 22, 23	Present
	Death	May 23	1.2 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1120 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00489	Hunched Posture Prostrate Increased Startle Reflex Squinting Twitching Tremors Chromodacyorrhea Death	May 22 May 22 May 22 May 22 May 22 May 22 May 22 May 23	Marked Present Slight Marked Marked Moderate Moderate 1 day
85D00494	Hypertonia Prostrate Increased Startle Reflex Squinting Twitching Irritable Hunched Posture Inactive Chromodacryorrhea Cyanosis Lacrimation Moribund Death	May 22 May 22 May 22 May 22, 23 May 22 May 22 May 22 May 22 May 22 May 23 May 23 May 23 May 23	Slight Present Marked Marked Marked Slight Marked Marked Moderate Slight Moderate Present 1.2 days
85D00503	Hunched Posture	May 22, 23	Slight
85D00505	Hypertonia Prostrate Increased Startle Reflex Squinting Twitching Chromodacryorrhea Death	May 22 May 22 May 22 May 22 May 22 May 22 May 23	Slight Present Slight Marked Marked Marked 1 day
85D00525	Misdose	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1260 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00460	Hypertonia	May 16, 17	Marked
	Prostrate	May 16	Present
	Squinting	May 16	Marked
	Twitching	May 16	Marked
	Cyanosis	May 16, 17	Moderate
	Moribund	May 16, 17	Present
	Death	May 18	2 days
85D00465	Prostrate	May 16	Present
	Increased Startle Reflex	May 16	Moderate
	Squinting	May 16	Marked
	Twitching	May 16	Marked
	Tremors	May 16	Marked
	Cyanosis	May 16	Slight
	Jumping	May 16	Moderate
	Moribund	May 16	Present
	Death	May 17	1 day
85D00474	Hypertonia	May 16	Moderate
	Prostrate	May 16	Present
	Increased Startle Reflex	May 16	Moderate
	Squinting	May 16	Marked
	Twitching	May 16	Slight
	Tremors	May 16	Marked
	Cyanosis	May 16	Slight
	Stain, Mouth, Red	May 16	Slight
	Death	May 17	1 day
85D00476	Misdose	N/A	N/A
85D00491	Depressed Righting Reflex	May 16	Marked
	Prostrate	May 16	Present
	Depressed Grasping Reflex	May 16	Moderate
	Squinting	May 16	Marked
	Twitching	May 16	Marked
	Tremors	May 16	Marked
	Cyanosis	May 16	Slight
	Jumping	May 16	Slight
	Moribund	May 16	Present
	Death	May 17	1 day

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1260 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00495	Prostrate Squinting Twitching Cyanosis Moribund Death	May 16 May 16 May 16 May 16 May 16 May 17	Present Marked Marked Moderate Present 1 day
85D00500	Misdose	N/A	N/A
85D00519	Hypertonia Prostrate Depressed Grasping Reflex Squinting Twitching Tremors Cyanosis Lacration Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Moderate Present Moderate Marked Marked Slight Slight Moderate 1 day
85D00521	Inactive Prostrate Depressed Grasping Reflex Squinting Twitching Tremors Cyanosis Moribund Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Moderate Present Moderate Marked Marked Slight Slight Present 1 day
85D00524	Hunched Posture Prostrate Squinting Twitching Tremors Cyanosis Moribund Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Moderate Present Marked Marked Marked Moderate Present 1 day

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 631 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00530	Misdose	N/A	N/A
85D00536	Hunched Posture	May 22-24	Moderate
	Inactive	May 22	Moderate
	Depressed Grasping Reflex	May 22	Marked
	Squinting	May 22	Marked
	Irritable	May 22	Slight
	Tremors	May 22	Slight
	Chromodacryorrhea	May 22	Marked
	Stain, Nose, Red	May 23, 24	Moderate
	Stains, Perianal	May 23-26	Marked
	Stain, Abdomen, Yellow	May 24	Marked
	Diarrhea	May 24	Slight
85D00537	Hunched Posture	May 22-25	Marked
	Inactive	May 22-25	Marked
	Depressed Grasping Reflex	May 22	Marked
	Hypertonia	May 22	Marked
	Ataxia	May 22, 23	Moderate
	Tremors	May 22-24	Marked
	Stain, Nose, Yellow	May 25	Marked
	Stain, Perianal, Yellow	May 23-25	Marked
	Stain, Abdomen, Yellow	May 24, 25	Marked
	Death	May 26	4 days
85D00547	Hunched Posture	May 22, 23	Moderate
	Inactive	May 22	Slight
	Depressed Grasping Reflex	May 22	Slight
	Irritable	May 23	Moderate
	Chromodacryorrhea	May 22	Slight
	Stain, Nose, Red	May 22	Slight
	Stains, Perianal	May 23	Moderate
	Diarrhea	May 22, 23	Marked
85D00561	Hunched Posture	May 22, 23	Moderate
	Inactive	May 22	Slight
	Irritable	May 23	Slight
	Stain, Perianal, Yellow	May 23	Marked
	Diarrhea	May 22	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**FEMALE: 631 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)**

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00565	Hunched Posture Inactive Irritable Stains, Perianal	May 22, 23 May 22 May 22-24 May 22	Moderate Slight Moderate Moderate
85D00568	Hunched Posture Inactive Irritable Hypertonia Stain, Perianal, Yellow Stain, Eye, Brown	May 22, 23 May 22 May 22 May 22 May 23 May 28-30	Marked Slight Slight Slight Slight Slight
85D00569	Hunched Posture Inactive Hypertonia Ataxia	May 22, 23 May 22 May 22 May 22	Slight Slight Slight Slight
85D00579	Hunched Posture Inactive Increased Startle Reflex Irritable Stains, Perianal	May 22-26 May 22-24 May 22 May 22 May 23-25	Marked Marked Slight Slight Marked
85D00581	Hunched Posture Inactive Squinting Irritable Lacrimation Stain, Nose/Mouth, Red Stain, Perianal, Yellow Hypertonia	May 22, 23 May 22, 23 May 22 May 23, 24 May 22 May 22 May 23 May 22	Moderate Slight Slight Moderate Slight Slight Marked Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 794 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00529	Misdose	N/A	N/A
85D00532	Irritable	May 23	Slight
85D00539	Hunched Posture	May 22-26	Marked
	Inactive	May 23, 26	Marked
	Hypertonia	May 22	Slight
	Squinting	May 26	Marked
	Irritable	May 24	Slight
	Tremors	May 22, 23	Slight
	Ataxia	May 22, 23	Moderate
	Stains, Perianal	May 23-26	Marked
	Stain, Abdomen, Yellow	May 24-26	Marked
	Diarrhea	May 25, 26	Moderate
	Increased Resp. Rate	May 24	Slight
	Death	May 27	5 days
85D00541	Inactive	May 15	Marked
	Depressed Grasping Reflex	May 15	Marked
	Squinting	May 15	Moderate
	Prostrate	May 15	Present
	Tremors	May 15	Slight
	Twitching	May 16	Slight
	Stains, Perianal	May 16	Slight
	Moribund	May 16	Present
	Death	May 17	2 days
85D00543	Hunched Posture	May 23	Marked
	Inactive	May 22	Marked
	Depressed Grasping Reflex	May 22	Marked
	Squinting	May 22, 23	Marked
	Prostrate	May 22	Present
	Tremors	May 22	Moderate
	Twitching	May 22	Slight
	Chromodacryorrhea	May 22	Marked
	Stain, Nose, Red	May 22	Slight
	Increased Startle Reflex	May 22	Slight
	Stain, Mouth	May 23	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 794 mg/kg DIETHYLENEGLCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00543 (cont.)	Lacrimation	May 23	Marked
	Cyanosis	May 23	Slight
	Moribund	May 23	Present
	Death	May 23	1.2 days
85D00551	Misdose	N/A	N/A
85D00552	Hunched Posture	May 22-26	Marked
	Inactive	May 22	Moderate
	Hypertonia	May 22	Slight
	Tremors	May 23, 24	Moderate
	Diarrhea	May 23	Moderate
	Increased Startle Reflex	May 22	Moderate
	Stain, Perianal, Yellow	May 23-27	Marked
	Stain, Abdomen, Yellow	May 26	Marked
85D00553	Hunched Posture	May 22-24	Marked
	Inactive	May 23-24	Marked
	Depressed Grasping Reflex	May 22	Marked
	Squinting	May 22	Slight
	Twitching	May 22	Slight
	Tremors	May 22, 23	Slight
	Ataxia	May 22	Moderate
	Stains, Perianal	May 23, 24	Marked
	Stain, Abdomen, Yellow	May 24	Marked
	Lacrimation	May 24	Marked
	Death	May 25	3 days
85D00555	Hunched Posture	May 22-24	Moderate
	Inactive	May 22, 24	Moderate
	Increased Startle Reflex	May 22	Moderate
	Squinting	May 22, 23	Moderate
	Hypertonia	May 22	Slight
	Prostrate	May 22, 24	Present
	Twitching	May 22	Marked
	Tremors	May 23, 24	Marked
	Ataxia	May 22	Moderate
	Urine, Dark	May 23	Marked

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 794 mg/kg DIETHYLENEMEGYLCOOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00555 (cont.)	Stain, Nose, Red	May 23	Slight
	Lacrimation	May 23	Marked
	Stain, Perianal, Dark	May 23, 24	Marked
	Stain, Abdomen, Yellow	May 24	Slight
	Death	May 24	2.2 days
85D00559	Hunched Posture	May 22-24	Marked
	Inactive	May 22-24	Moderate
	Increased Startle Reflex	May 22	Slight
	Depressed Grasping Reflex	May 22	Marked
	Squinting	May 22-24	Marked
	Prostrate	May 23, 24	Marked
	Tremors	May 22	Moderate
	Ataxia	May 22-24	Moderate
	Chromodacryorrhea	May 22	Marked
	Lacrimation	May 23, 24	Marked
85D00566	Death	May 25	3 days
	Hunched Posture	May 15-17	Marked
	Inactive	May 15, 16	Marked
	Depressed Grasping Reflex	May 15, 16	Marked
	Squinting	May 15, 16	Marked
	Tremors	May 15	Slight
	Stains, Perianal	May 16, 17	Marked
	Stains, Abdomen	May 16, 17	Marked
	Diarrhea	May 16	Marked
	Rough Coat	May 16	Moderate
	Cyanosis	May 16	Slight
	Decreased Resp. Depth	May 16	Present
	Increased Resp. Rate	May 17	Moderate
85D00571	Moribund	May 17	Present
	Death	May 17	2.2 days
85D00572	Misdose	N/A	N/A
85D00572	Misdose	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 794 mg/kg DIETHYLENEGLCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00574	Inactive Hunched Posture Stain, Perianal, Yellow	May 15 May 15 May 16	Slight Slight Moderate
85D00590	Misdose	N/A	N/A
85D00592	Misdose	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 891 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00534	Hunched Posture Inactive Increased Startle Reflex Hypertonia Irritable	May 22, 23 May 22 May 22 May 22 May 22-24, 28, 29	Moderate Slight Slight Moderate Marked
85D00538	Misdose	N/A	N/A
85D00545	Increased Startle Reflex Squinting Prostrate Twitching Tremors Chromodacryorrhea Moribund Death	May 22 May 22 May 22 May 22 May 22 May 22 May 22 May 23	Slight Marked Present Marked Slight Moderate Present 1 day
85D00562	Inactive Increased Startle Reflex Squinting Prostrate Twitching Tremors Chromodacryorrhea Lacration Death	May 22 May 22 May 22 May 22 May 22 May 22 May 22 May 23	Marked Slight Marked Marked Marked Moderate Moderate Marked 1 day
85D00564	Misdose	N/A	N/A
85D00576	Inactive Depressed Righting Reflex Depressed Grasping Reflex Squinting Prostrate Twitching Tremors Chromodacryorrhea Lacration Cyanosis	May 22 May 22 May 22 May 22, 23 May 22 May 22 May 22 May 22 May 22 May 23	Marked Slight Moderate Marked Present Moderate Moderate Moderate Marked Marked

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 891 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00576 (cont.)	Increased Resp. Depth Moribund Death	May 23 May 23 May 24	Slight Present 1.2 days
85D00582	Hunched Posture Inactive Squinting Hypertonia Prostrate Stain, Head, Red Lacrimation Stain, Perianal, Brown Wheezing Cyanosis Moribund Death	May 22 May 22 May 22 May 22 May 22 May 22 May 22, 23 May 23 May 23 May 23 May 23 May 24	Slight Slight Slight Slight Present Slight Marked Marked Slight Slight Present 2 days
85D00587	Hunched Posture Inactive Increased Startle Reflex Depressed Grasping Reflex Squinting Hypertonia Twitching Tremors Chromodacryorrhea Stain, Perianal, Green Cyanosis Moribund Death	May 22 May 22 May 22 May 22 May 22, 23 May 22 May 22 May 22 May 22, 23 May 23 May 23 May 23 May 23	Marked Marked Marked Moderate Marked Moderate Moderate Slight Marked Slight Slight Present 1.2 days
85D00593	Hunched Posture Inactive Increased Startle Reflex Depressed Grasping Reflex Squinting Prostrate Twitching Tremors Chromodacryorrhea Death	May 22 May 22 May 22 May 22 May 22 May 22 May 22 May 22 May 22 May 23	Slight Marked Marked Moderate Marked Present Moderate Marked Moderate 1 day

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 891 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00595	Increased Startle Reflex	May 22	Slight
	Squinting	May 22, 23	Marked
	Prostrate	May 22, 23	Marked
	Twitching	May 22, 23	Slight
	Lacrimation	May 22, 23	Marked
	Moribund	May 23	Marked
	Cyanosis	May 23	Moderate
	Death	May 23	1.2 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1000 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00531	Increased Startle Reflex Squinting Prostrate Twitching Tremors Lacrimation Cyanosis Moribund Death	May 16 May 16 May 16 May 16 May 16 May 16, 17 May 16, 17 May 16, 17 May 17	Slight Marked Marked Moderate Moderate Moderate Moderate Slight 1.1 days
85D00546	Misdose	N/A	N/A
85D00559	Increased Startle Reflex Squinting Hypertonia Prostrate Twitching Tremors Lacrimation Cyanosis Pallor Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Marked Marked Slight Present Moderate Moderate Marked Slight Slight 1 day
85D00556	Hunched Posture Inactive Increased Startle Reflex Squinting Hypertonia Twitching Irritable Lacrimation Cyanosis Moribund Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17 May 17 May 17 May 18	Moderate Slight Slight Marked Slight Moderate Slight Marked Moderate Present 2 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1000 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00558	Hunched Posture Increased Startle Reflex Squinting Prostrate Twitching Tremors Jumping Lacration Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Marked Slight Marked Present Slight Moderate Moderate Slight 1 day
85D00563	Squinting Jumping Prostrate Twitching Tremors Lacration Cyanosis Pallor Moribund Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Marked Slight Present Marked Moderate Slight Slight Slight Present 1 day
85D00567	Misdose	N/A	N/A
85D00570	Hunched Posture Inactive Increased Startle Reflex Depressed Grasping Reflex Irritable	May 16 May 16 May 16 May 16 May 16	Slight Slight Slight Moderate Slight
85D00575	Hunched Posture Inactive Squinting Hypertonia Jumping Twitching Lacration Cyanosis Moribund Death	May 16 May 16 May 16 May 16 May 16 May 17 May 16, 17 May 17 May 17 May 17	Moderate Marked Slight Slight Moderate Moderate Marked Moderate Present 1.2 days
85D00585	Misdose	N/A	N/A

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1260 mg/kg DIETHYLENEGLYCOL DINITRATE

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00559	Hunched Posture Inactive Increased Startle Reflex Squinting Hypertonia Prostrate Irritable Twitching Lacrimation Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Marked Moderate Slight Slight Moderate Present Slight Moderate Moderate 1 day
85D00542	Hunched Posture Inactive Squinting Prostrate Twitching Tremors Lacrimation Moribund Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Moderate Moderate Marked Present Moderate Moderate Moderate Present 1 day
85D00544	Misdose	N/A	N/A
85D00549	Depressed Righting Reflex Squinting Hypertonia Prostrate Twitching Tremors Jumping Cyanosis Lacrimation Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Moderate Marked Slight Present Marked Marked Moderate Slight Moderate 1 day
85D00550	Death	May 16	1.3 hours

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1260 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00554	Squinting Prostrate Twitching Tremors Stain, Nose, Red Lacrimation Death	May 16 May 16 May 16 May 16 May 16 May 16 May 17	Marked Present Marked Moderate Slight Slight 1 day
85D00580	Squinting Prostrate Twitching Tremors Lacrimation Cyanosis Death	May 16 May 16 May 16 May 16 May 16 May 16 May 17	Marked Present Slight Marked Marked Slight 1 day
85D00583	Misdose	N/A	N/A
85D00584	Hunched Posture Squinting Prostrate Twitching Tremors Stain, Mouth, Clear Lacrimation Cyanosis Death	May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 16 May 17	Slight Marked Present Moderate Slight Slight Moderate Slight 1 day
85D00588	Misdose	N/A	N/A

Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS**794 mg/kg MALES**

Animal No.	Receipt	Dosing	Day 9	Day 14
85D00470	146	201	241	256
85D00471	159	219	290	292
85D00484	150	218	294	294
85D00496	151	226	290	288
85D00497	154	220	276	271
85D00513	149	217	284	284
85D00517	148	213	279	275

Mean	151.0	216.3	279.1	280.0
Standard Deviation	4.32	7.78	18.00	13.55
Std. Error of Mean	1.63	2.94	6.81	5.12

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**891 mg/kg MALES**

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00459	153	255	292	296
85D00467	155	254	299	298
85D00481	165	267	Dead	
85D00502	154	242	278	275
85D00504	146	226	252	261
85D00506	140	227	Dead	
85D00520	152	262	318	322
85D00527	145	254	317	311
<hr/>				
Mean	151.3	248.4	292.7	293.8
Standard Deviation	7.63	15.30	25.10	22.55
Std. Error of Mean	2.70	5.41	10.24	9.21

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**1000 mg/kg MALES**

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00464	155	218	279	286
85D00468	158	220	Dead	
85D00477	149	213	Dead	
85D00478	157	217	249	285
85D00483	163	227	Dead	
85D00487	160	235	272	307
85D00501	160	219	292	276
<hr/>				
Mean	157.4	221.3	273.0	288.5
Standard Deviation	4.50	7.36	18.02	13.13
Std. Error of Mean	1.70	2.78	9.01	6.56

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**1120 mg/kg MALES**

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00466	162	264	Dead	
85D00469	150	263	Dead	
85D00473	155	270	Dead	
85D00488	151	279	Dead	
85D00489	148	242	Dead	
85D00494	152	262	Dead	
85D00503	147	272	336	340
85D00505	161	278	Dead	
<hr/>				
Mean	153.3	266.3		
Standard Deviation	5.65	11.77		
Std. Error of Mean	2.00	4.16		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**1260 mg/kg MALES**

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00460	164	223	Dead	
85D00465	154	213	Dead	
85D00474	151	209	Dead	
85D00491	144	205	Dead	
85D00495	145	199	Dead	
85D00519	165	228	Dead	
85D00521	156	228	Dead	
85D00524	124	198	Dead	
<hr/>				
Mean	150.4	212.9		
Standard Deviation	13.15	12.25		
Std. Error of Mean	4.65	4.33		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**Control MALES**

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00485	155	267	320	317
85D00498	151	258	311	306
85D00510	148	263	320	321
85D00516	151	247	314	312
85D00526	155	266	333	329
<hr/>				
Mean	152.0	260.2	319.6	317.0
Standard Deviation	3.00	8.17	8.44	8.75
Std. Error of Mean	1.34	3.65	3.78	3.91

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**631 mg/kg FEMALES**

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00536	160	202	242	237
85D00537	157	202	Dead	
85D00547	151	187	220	209
85D00561	166	222	250	251
85D00565	158	203	228	228
85D00568	164	217	263	245
85D00569	155	203	237	225
85D00579	163	204	237	235
85D00581	156	205	237	227
<hr/>				
Mean	158.9	205.0	239.3	232.1
Standard Deviation	4.81	9.90	13.09	13.00
Std. Error of Mean	1.60	3.30	4.63	4.60

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**794 mg/kg FEMALES**

Animal No.	Receipt	Dosing	Day 3	Day 14
85D00532	165	195	195	209
85D00539	174	221	Dead	
85D00541	160	179	Dead	
85D00543	152	188	Dead	
85D00552	159	203	233	236
85D00553	161	212	Dead	
85D00555	167	198	Dead	
85D00559	170	196	Dead	
85D00566	160	183	Dead	
85D00574	172	206	246	236
<hr/>				
Mean	164.0	198.1	224.7	227.0
Standard Deviation	6.83	12.97	26.50	15.59
Std. Error of Mean	2.16	4.10	15.30	9.00

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**891 mg/kg FEMALES**

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00534	153	192	231	224
85D00545	160	214	Dead	
85D00562	165	204	Dead	
85D00576	170	236	Dead	
85D00582	171	240	Dead	
85D00587	151	210	Dead	
85D00593	160	216	Dead	
85D00595	148	205	Dead	
<hr/>				
Mean	159.8	214.6		
Standard Deviation	8.61	16.22		
Std. Error of Mean	3.05	5.74		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

1000 mg/kg FEMALES

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00531	157	188	Dead	
85D00548	154	179	Dead	
85D00556	157	168	Dead	
85D00558	162	197	Dead	
85D00563	161	188	Dead	
85D00570	165	181	213	209
85D00575	147	170	Dead	
- - - - -				
Mean	157.6	181.6		
Standard Deviation	5.94	10.37		
Std. Error of Mean	2.25	3.92		

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**1260 mg/kg FEMALES**

Animal No.	Receipt	Dosing	Day 8	Day 14
85D00540	171	187	Dead	
85D00542	168	192	Dead	
85D00549	159	188	Dead	
85D00550	172	200	Dead	
85D00554	160	195	Dead	
85D00580	162	188	Dead	
85D00584	165	194	Dead	
<hr/>				
Mean	165.3	192.0		
Standard Deviation	5.22	4.73		
Std. Error of Mean	1.97	1.79		

Appendix G: Pathology Report

LAIR Pathology Report
GLP Study 84017

Investigator: MAJ Larry D. Brown.

History: This study was conducted in accordance with SOP-OP-STX-36. It was conducted in young, mature Sprague-Dawley Albino rats. The test compound diethylene glycol, dinitrate (DEGDN), (CAS No. 693-21-0). The dose vehicle was corn oil. Male and female were divided into each of the following dosage group and treatment levels:

DOSAGE GROUP	SEX	DOSE LEVEL <u>(mg DEGDN/kg BW)</u>
1	Male and Female	794
2	Male and Female	1000
3	Male and Female	1260
4	Male and Female	891
5	Male only	1120
6	Male only	0
7	Female only	631

Gross Necropsy Results (Males): The individual animal gross findings are as follows:

DOSE GROUP 1 - 794 mg/kg
MALES

LAIR ACCESSION#	ID#	GROSS FINDINGS
37668	85D00470	Live - Hydronephrosis
37669	85D00471	Live - Not remarkable (NR)
37670	85D00484	Live - NR
37671	85D00496	Live - NR
37672	85D00497	Live - NR
37673	85D00513	Live - NR
37674	85D00517	Live - NR

Appendix G (cont.): Pathology Report

Pathology Report
GLP Study 84017

DOSE GROUP 2 - 1000 mg/kg
MALES

LAIR ACCESSION#	ID#	GROSS FINDINGS
37677	85D00464	Live - NR
37584	85D00468	Dead - pale liver and kidney, missing ear tag
37560	85D00477	Dead - yellow fluid in stomach and intestine
37678	85D00478	Live - NR
37561	85D00483	Dead - yellow fluid in stomach and intestine
37679	85D00487	Live - NR
37680	85C00501	Live - NR

DOSE GROUP 3 - 1260 mg/kg
MALES

37583	85D00460	Dead - pale liver
37557	85D00465	Dead - yellow fluid in stomach, intestine.
37558	85D00474	Dead - yellow fluid in stomach and intestine
37562	85D00491	Dead - yellow fluid in stomach and intestine
37564	85D00495	Dead - red material on muzzle and yellow fluid in stomach and intestine
37565	85D00519	Dead - yellow fluid in stomach and intestine
37566	85D00521	Dead - yellow fluid in stomach and intestine
37567	85D00524	Dead - yellow fluid in stomach and intestine

Appendix G (cont.): Pathology Report

Pathology Report
GLP Study 84017

DOSE GROUP 4 - 891 mg/kg
MALES

LAIR ACCESSION#	ID#	GROSS FINDINGS
37777	85D00459	Live - NR
37778	85D00467	Live - NR
37658	85D00481	Dead - hydronephrosis, pale liver
37781	85D00502	Live - NR
37783	85D00504	Live - NR
37663	85D00506	Dead - severe autolysis
37786	85D00520	Live - NR
37788	85D00527	Live - NR

DOSE GROUP 5 - 1120 mg/kg
MALES

37645	85D00466	Dead - NR
37646	85D00469	Dead - NR
37639	85D00473	Dead - missing ear tag
37647	85D00488	Dead - NR
37640	85D00489	Dead - NR
37648	85D00494	Dead - NR
37782	85D00503	Live - mass in peritoneal cavity
37641	85D00505	Dead - NR

DOSE GROUP 6 - 0 mg/kg
MALES

37779	85D00485	Live - yellow focus in epididymus, white foci in liver and missing ear tag
37780	85D00498	Live - Testicle atrophy
37784	85D00510	Live - NR
37785	85D00516	Live - NR
37787	85D00526	Live - NR

Appendix G (cont.): Pathology Report

Pathology Report
GLP Study 84017

SUMMARY TABLE - MALE

Group No.	1	2	3	4	5	6
Dose (mg/kg)	794	1000	1260	891	1120	0
Animals/Group	7	7	8	8	8	5
Death	0	3	8	2	7	0
% Death	0	42.8	100	25	87.5	0
Survivor	7	4	0	6	1	5
Not Remarkable	6	4	0	6	6	3
GI Fluid	0	2	7	0	0	0
Pale Liver	0	1	1	1	0	0
Pale Kidney	0	1	0	0	0	0
Hydronephrosis	1	0	0	1	0	0
Missing ear tag	0	1	0	0	1	1
Red nasal material	0	0	1	0	0	0
Autolysis	0	0	0	1	0	0
Epididymal focus	0	0	0	0	0	1
Liver foci	0	0	0	0	0	1
Peritoneal mass	0	0	0	0	1	0
Testicle atrophy	0	0	0	0	0	1

Deaths occurred up to four days after dosing. All survivors were killed by sodium pentobarbital injection two weeks after dosing. The only gross change that appears to be related to treatment is the presence of yellow oily fluid in the stomach and upper small intestine.

Appendix G (cont.): Pathology Report

Pathology Report
GLP Study 84017

Gross Necropsy Results (Females): The individual animal gross findings are as follows:

DOSE GROUP 1 - 794 mg/kg
FEMALES

LAIR ACCESSION #	ID #	GROSS FINDINGS
37675	85D00532	Live - NR
37665	85D00539	Dead - severe autolysis
37569	85D00541	Dead - petechial hemorrhage of stomach and pale liver
37649	85D00543	Dead - NR
37792	85D00552	Live - NR
37666	85D00553	Dead - severe autolysis
37659	85D00555	Dead - pale liver and kidney, petechial hemorrhage, brown liquid in stomach and intestine
37667	85D00559	Dead - severe autolysis
37580	85D00566	Dead - petechial hemorrhage; dark material in intestine; pale liver and brownish urine
37676	85D00574	Live - NR

DOSE GROUP 2 - 1000 mg/kg
FEMALES

37578	85D00531	Dead - petechial hemorrhage in stomach
37571	85D00548	Dead - yellow fluid in stomach and intestine
37585	85D00556	Dead - fluid in stomach and intestine; pale liver
37574	85D00558	Dead - yellow fluid in stomach and intestine
37575	85D00563	Dead - yellow fluid in stomach and intestine
37681	85D00570	Live - NR
37579	85D00575	Dead - petechial hemorrhage in stomach; pale liver; brownish urine

Appendix G (cont.): Pathology Report

Pathology Report
GLP Study 84017

DOSE GROUP 3 - 1260 mg/kg
FEMALES

LAIR ACCESSION #	ID #	GROSS FINDINGS
-------------------------	-------------	-----------------------

37568	85D00540	Dead - yellow fluid in stomach and intestine; missing ear tag
37570	85D00542	Dead - yellow fluid in stomach and intestine; red material on muzzle
37572	85D00549	Dead - yellow fluid in stomach and intestine
37552	85D00550	Dead - NR
37573	85D00554	Dead - yellow fluid in stomach and intestine
37576	85D00580	Dead - corneal opacity and yellow fluid in stomach and intestine
37577	85D00584	Dead - yellow fluid in stomach and intestine

DOSE GROUP 4 - 891 mg/kg
FEMALES

37789	85D00534	Live - ear tag missing
37642	85D00545	Dead - NR
37643	85D00562	Dead - NR
37650	85D00576	Dead - NR
37654	85D00582	Dead - petechial hemorrhage in stomach
37651	85D00587	Dead - brownish urine
37644	85D00593	Dead - NR
37652	85D00595	Dead - brownish urine

Appendix G (cont.): Pathology Report

Pathology Report
GLP Study 84017

DOSE GROUP 7 - 631 mg/kg
FEMALES

LAIR ACCESSION #	ID #	GROSS FINDINGS
37790	85D00536	Live - NR
37664	85D00537	Dead - severe autolysis
37791	85D00547	Live - NR
37793	85D00561	Live - NR
37794	85D00565	Live - NR
37795	85D00568	Live - NR
37796	85D00569	Live - NR
37797	85D00579	Live - NR
37776	85D00581	Live - NR

SUMMARY TABLE - FEMALES

Group No.	1	2	3	4	7
Dose (mg/kg)	794	1000	1260	891	631
Animals/Group	10	7	7	8	9
Death	7	6	7	7	1
% Death	70	85.7	100	87.5	11.1
Survivor	3	1	0	1	8
Not Remarkable	4	1	1	4	8
Autolysis	3	0	0	0	1
GI Hemorrhage	3	2	0	1	0
GI fluid	1	4	6	0	0
Pale Liver	3	2	0	0	0
Pale Kidney	1	0	0	0	0
Corneal opacity	0	0	1	0	0
Brown Urine	1	1	0	2	0
Missing ear tag	0	0	1	1	0
Red nasal material	0	0	1	0	0

Deaths occurred up to five days after dosing. All survivors were killed by sodium pentobarbital injection two weeks after dosing. Gross findings that may be related to treatment include hemorrhage and fluid accumulation in the stomach and intestine and possibly brownish discoloration of urine.

Appendix G (cont.): Pathology Report

Pathology Report
GLP Study 84017

Microscopic Findings: The individual animal microscopic findings are as follows:

LAIR	ACC#	ID#	GROUP	SEX	MICROSCOPIC FINDINGS
37583	85D00460	3	M		Liver vacuolization and renal autolysis
37584	85D00468	2	M		Liver vacuolization
37779	85D00485	6	M		Liver necrosis and perm granuloma
37782	85D00503	5	M		Bacterial granuloma
37569	85D00541	1	F		Liver vacuolization, gastritis
37659	85D00555	1	F		Liver vacuolization, gastritis and lymphoid necrosis of gut
37585	85D00556	2	F		Liver vacuolization
37580	85D00566	1	F		Liver vacuolization, renal necrosis, gastritis and lymphoid necrosis and apoptosis of gut
37579	85D00575	2	F		Liver vacuolization and gastritis
37576	85D00580	3	F		Autolysis of eye

SUMMARY TABLE - MALE AND FEMALE POOLED

Group No.	1	2	3	5	6
Dose (mg/kg)	794	1000	1260	1120	0
Liver Vacuolization	3	3	1	0	0
Gastritis	3	1	0	0	0
Lymphoid necrosis	1	0	0	0	0
Renal necrosis	1	0	0	0	0
Renal autolysis	0	0	1	0	0
Peritoneal granuloma	0	0	0	1	0
Sperm granuloma	0	0	0	0	1
Liver necrosis	0	0	0	0	1

The multifocal necrohemorrhagic gastritis that was observed in groups 1 and 2 is probably treatment related. Liver vacuolization is a nonspecific diagnosis and may or may not be related to treatment. The renal autolysis was limited to proximal tubules, raising the possibility of treatment established from the available specimens because of uncertainty of time between death and necropsy. Other lesions noted are probably incidental and unrelated to treatment.

Appendix G (cont.): Pathology Report

Pathology Report
GLP Study 84017

Result Summary: A dose response effect on deaths is apparent in both male and female rats. Microscopic evaluation of gross necropsy lesions indicates that stomach is a probable target tissue. Liver, kidney, gut and lymphoid tissue should be considered as potential target tissues for any future microscopic studies.

for Charles S. Coffey, DVM, PhD 
for ROBERT L. MORRISSEY, DVM LANCE O. LOLLIINI, DVM
LTC, VC LTC, VC
USAR Chief, Pathology Services Group

2 August 1985

Distribution List

Commander

US Army Biomedical Research and
Development Laboratory (27)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center
(DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and
Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEEHA Regional Division, South
Bidg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commander US Army Materiel
Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander

US Army Environmental Hygiene
Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean

School of Medicine
Uniformed Services University of the
Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMcen-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314

CDR, US Army Toxic and Hazardous
Material Agency
ATTN: DRXTH/ES
Aberdeen Proving Ground, MD 21010

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental
Quality Branch
Preventive Medicine Division
(HSHA-IPM)
Fort Sam Houston, TX 78234